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Gender Bias in Haemato-Biochemical and Oxidative Stress Indices in Karakul Sheep

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ABSTRACT

Twelve apparently healthy 3-4 year old animals from cold arid deserts of Ladakh region, with equal representation of male and female were bled in the jugular vein after observing strict aseptic measures. Three samples from each animal were collected at an interval of 15 days and values averaged. Normal physiological levels of haemato-biochemical parameters and oxidative stress markers were recorded. Many of the hematological parameters did differ between sexes. Total erythrocyte count, total leukocyte count and haemoglobin was significantly ($P < 0.05$) higher (10.32×10^6, 9.03×10^3 and 11.25 g/dl) in males as compared to females (9.04×10^6, 7.75×10^3 and 10.10 g/dl) respectively. Similarly plasma albumin (3.53 g/dl) and creatinine (1.28 mg/dl) was significantly ($P < 0.05$) higher and cholesterol (77.66 mg/dl) significantly ($P < 0.01$) lower in males as compared to females (3.23 g/dl, 1.03 mg/dl and 113.83 mg/dl) respectively. Antioxidant power determined by FRAP did not reveal any significant difference between sexes, however, TBARS differed significantly ($P < 0.01$) between the sexes with higher value (3.84 ± 0.15 nM/L) in males than females (2.88 ± 0.22 nM/L). Various other plasma analytes including liver enzymes did not vary significantly between the sexes. Present study recognized the course of various blood constituents between the sexes of Karakul sheep which was not available in literature and thus can prove potentially valuable diagnostic tool in the health care and clinical interpretation of laboratory data in these animals.

Keywords: Haemato-biochemical indices, karakul sheep, oxidative stress markers, sex bias

The Karakul, known world-wide for its fur, wool and meat, is also referred as fur sheep. Karakul is possibly the oldest breed of domesticated sheep and exceptionally hardy, intelligent, with good mothering instincts, milkiness, good feet, long legs for traveling, resistance to disease and characteristic tail for fat storage. Its lamb pelt with intricately patterned curls is very famous, costlier, used for making caps (Karakuli) and other garments. The Karakul is native to Central Asia and is named after a village called Karakul. In Jammu and Kashmir State Karakul is found in cold arid climate of Ladakh region thriving at an altitude of 3000-4000 meters above mean sea level (amsl) under very harsh conditions of chilling cold with minimum temperature dipping to -20 ^oC during winter. Very few small flocks of Karakul presently exist in the Kargil area of Ladakh region and State government is taking necessary measures for conservation of this gorgeous and hardy breed. Blood is of crucial importance for the maintenance of physiological equilibrium in the body and provides an opportunity to clinically investigate the presence of metabolites and other constituents in the animals for clinical interpretation and understanding of physiological, nutritional and pathological status of an animal (Dutta *et al*., 1988; NseAbasi *et al*., 2014) certain blood biochemical constituents reflect the nutritional status/metabolic health of livestock with, or without, the manifestation of clinical abnormalities (Roubiens *et al*., 2006). Haemato-biochemical analysis and evaluation of thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of plasma (FRAP) in animals could provide complementary information about the homeostasis of the animal than conventional metabolic parameters alone (Pampori and Pandita, 2013). There stands a good amount of studies that reveal sex bias in the prevalence

of many bacterial and parasitic diseases in domestic animals (Rajput *et al*., 2005; Ramazan *et al*., 2009). Taking cognizance of gender bias in animal pathologies and blood as an important biological material, present study was undertaken in distinct breed of sheep to record and recognize the gender bias in haemato-biochemical parameters. The knowledge of normal physiological blood parameters and interpretation of clinical data becomes an effective tool for application of medical intervention in the management of livestock disorders.

MATERIALS AND METHODS

Twelve apparently healthy 3-4 year old Karakul sheep, with equal representation of male and female, from Kargil district of cold arid Ladakh region were taken for present study during the month of August-September. The body dimensions and the weight of the animals was recorded using standard measuring tapes and balance. The animals were bled in the jugular vein after observing strict aseptic measures and 5 ml blood was collected in heparinized vacutainers. Blood smears were prepared on spot and samples were taken on ice to the laboratory. Three blood samples from each animal at an interval of 15 days were taken for present study and values averaged for each animal. All hematological studies were completed within 48 hours of collection and plasma was separated by centrifugation at 3000 rpm for 30 minutes. Haemoglobin (Hb) and packed cell volume (PCV) were estimated by cyanmethemoglobin method and micro hematocrit method, respectively. Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined by using Neubaur's haemocytometer. Erythrocyte fragility rate was determined by suspending the red blood cells in serially diluted saline (0.9% to 0.25%). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated (Coles, 1986; Douglas and Wardrop, 2010). Cytometry was done using ocular micrometer.

Plasma was evaluated for a wide range of analytes like total protein (TP), albumin, urea, creatinine, triglycerides, cholesterol and enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The plasma analysis was carried in Interga-400 plus and Cobas-C11 automatic biochemistry analysers from Germany using Roche diagnostic kits.

Thiobarbituric acid reactive substance (TBARS), an index of oxidative stress, was evaluated by the method described by Asakawa and Matsushita (1980). The ferric reducing ability of plasma (FRAP), a measure of antioxidant power, was determined by a method described by Benzie and Strain (1996).

Mean values and standard errors were calculated and the results were treated statistically using student's t-test assessing the mutual statistical differences between the sexes (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

The Karakul sheep taken for present study were having different fleece colours viz. black, red, pink and grey with males horned and females polled. The average body length and height recorded in males was 31.16 ± 0.52 and 29.56 ± 0.62 inches and in females 26.58 ± 0.47, 25.41 ± 0.62 inches respectively. Body weight recorded in males and females was 72.45 ± 1.90 and 54.73 ± 1.52 kg respectively. The body length and body weight differed significantly ($P < 0.01$) between the sexes. The sex bias has been reported in body weight by many workers (Koch *et al*., 1959) and males remained heavier than females as is true with the present findings.

The values recorded in present study for haematological parameters are presented in Table 1. The haemoglobin, total erythrocyte count and total leucocyte count did present statistically significant ($P < 0.01$) difference between the sexes with higher values in males as compared to females. The values for various haematological parameters recorded in Karakul sheep either in males or females fall within the reference range for sheep (Douglas and Wardrop, 2010; Aleksandar *et al*., 2011). However, the reference values for haematological parameters of sheep described by Research Animal Resource (2009) are on lower side when equated with the present findings in Karakul sheep. Egbe-Nwiyi *et al*. (2000) revealed the influence of sex and age on RBC, WBC, HB and PCV and MCHC in Nigerian sheep that completely agrees with the present findings in Karakul sheep. The significant sex difference in Hb, TEC and TLC with higher values in males than females recorded in Karakul sheep during present study stands in total agreement with the findings of Tambuwal *et al*. (2002) in Red Sokoto goats of Nigeria and Addass *et al*. (2010) in indigenous sheep breeds of Nigeria. In contrast

to the present findings, Rabee *et al*. (2014) reported no significant difference in Hb, PCV and ESR between sexes rather found significant difference between age and breeds in different indigenous sheep breeds of Iraq. The values for haematological parameters in Somali sheep were on higher side (Saad and Sabry, 2014) but comparable to the native ewes of Odisha (Chiranjib *et al*., 2015) when compared to present findings in Karakul sheep.

Table 1: Comparative haematology of male and female Karakul Sheep

Blood Parameter	Male	Female
Haemoglobin (g/ dl)	11.25 ± 0.42**	10.10 ± 0.28**
PCV (%)	45.03 ± 1.0	43.36 ± 1.1
TEC (million/cumm)	10.32 ± 0.56*	09.04 ± 0.19*
TLC (thousand/cumm)	9.03 ± 0.49**	7.75 ± 0.29**
ESR (mm/hr)	1.11 ± 0.08	0.91 ± 0.08
MCV (fl)	44.14 ± 2.15	48.08 ± 1.80
MCH (pg)	10.96 ± 0.38	11.21 ± 0.49
MCHC (g%)	25.03 ± 1.08*	23.35 ± 0.84*
RBC diameter (µ)	4.99 ± 0.06	4.88 ± 0.05
Minimum RBC fragility (%saline)	0.48 ± 0.01	0.48 ± 0.01
Maximum RBC fragility (%saline)	0.42 ± 0.01	0.43 ± 0.01

Note: results are expressed as mean ± standard error.

*Values in the same row with superscript * (P < 0.05) and ** (P < 0.01) differ significantly.*

The variation in haematological values is governed by several factors including animal breed, gender, geographical distribution, parasitic infestation and health conditions. The Karakul sheep undertaken for the present study thrives at an altitude of 12000 ft amsl thus high haemoglobin concentration and erythrocyte count serves as an adaptation to high altitude to compensate relatively low atmospheric oxygen pressure there. Relatively low values in females as compared to males have been attributed to the metabolic status with females having low metabolic rate than males (Douglas and Wardrop, 2010). Higher leucocyte counts in males as compared to females reported in present study probably provide enhanced adaptability to varied stresses in males.

The plasma concentration of various analytes and enzymes recorded in Karakul sheep are presented in Table 2. There was no significant difference reported in total plasma proteins, triglycerides or liver enzymes between sexes.

Table 2: Comparative plasma analytes in male and female Karakul Sheep

Parameters	Male	Female
Total protein (g/dl)	6.75 ± 0.13	7.01 ± 0.13
Albumin (g/dl)	3.53 ± 0.1*	3.23 ± 0.07*
Globulin (g/dl)	3.21 ± 0.18**	3.78 ± 0.10**
A/G ratio	1.12 ± 0.08**	0.85 ± 0.03**
Triglycerides (mg/dl)	27.33 ± 3.53	26.66 ± 2.32
Cholesterol (mg/dl)	77.66 ± 5.45**	113.83 ± 5.59**
Urea (mg/dl)	45.50 ± 5.2	55.33 ± 7.6
Creatinine (mg/dl)	1.28± 0.07*	1.03 ± 0.11*
ALP (u/l)	100.00 ± 9.24	87.66 ± 6.99
AST (u/l)	156.83 ± 8.21	149.66 ± 4.48
ALT (u/l)	35.16 ± 8.12	38.50 ± 2.82
TBARS (nM/ml)	3.84 ± 0.15**	2.88 ± 0.22**
FRAP (uM/L)	415.50 ± 4.42	421.66 ± 2.39

Note: results are expressed as mean ± standard error.

*Values in the same row with superscript * (P < 0.05) and ** (P < 0.01) differ significantly.*

However, significant (P<0.01) sex difference was recorded in albumin/ globulin ratio, cholesterol and creatinine. Higher concentration of plasma globulin in Karakul ewes indicated indirectly presence of higher humoral resistance to diseases in ewes. Giltay *et al*. (2000) recorded higher serum levels of globulins in women than men that corroborate with the present findings in karakul ewes. The synthesis of albumin and globulin is taking place in the liver and these levels decrease in case of malnutrition, hepatic diseases, protein deficiency, starvation and malignancy (Keser and Bilal, 2008) as such analysis of these enzymes in plasma reflect the hepatic status of the subjects. Values reported for liver enzymes by Shumaila *et al*. (2012) in sheep of southern Punjab, Pakistan and Miloslav *et al*. (2011) in West African sheep were comparable to the values recorded in present study in Karakul sheep, whereas values reported by Perez *et al*. (2003) in wild goat were on higher side than the present findings, indicating a great variation in blood biochemical levels among different small ruminants having different geographical distribution. Mostaghni *et al*. (2005) in wild sheep of Iran, Mabruka (2014) in sheep of Libya and Khan

et al. (2013) in Karadi sheep of Iraq found no significant gender bias in the enzymes yet the values differed between sexes and these reports endorse the present findings in Karakul sheep in which the activity of ALT, AST, and ALP was not significantly influenced by gender. Present findings of biochemical parameters in karakul sheep are comparable to the findings of Saad and Sabry (2014) in Somali sheep and Chiranjib *et al*. (2015) in native ewes of Odisha.

In present study a significant ($P < 0.01$) effect of sex was found in plasma cholesterol concentration with higher levels in females as compared to males which is in contrast to the findings of Rabee *et al*. (2014) in indigenous sheep of Iraq. In similarity to present findings, Mabruka (2014) in sheep did record higher cholesterol levels in females than males but the difference was not statistically significant. Plasma cholesterol levels recorded in Karakul sheep were near to the levels reported by Devendra *et al.* (2008) in Coimbatore sheep and Jawasreh *et al.* (2010) in Awassi sheep.

Plasma urea levels in present study in Karakul sheep were within the reference range (Kaneko, 1989) but did not exhibit gender bias that stands in contrast to the findings of Mabruka (2014) who reported significantly higher plasma urea in males than females. However, findings of Rabee *et al*. (2014) in indigenous sheep of Iraq were in agreement with the present findings. Urea is an important metabolite synthesized from ammonia in the liver during protein metabolism and serves as an efficient indicator of kidney function (Keser and Bilal, 2008).The plasma creatinine levels reported in karakul sheep were within the reference range for sheep (Kaneko, 1989) but were higher than reported by Mabruka (2014) in sheep. The sex bias in the plasma creatinine with higher values in males as compared to females recorded in present study is in agreement with the findings of Mabruka (2014). The amount of creatinine secreted daily is a function of the muscle mass and lower values for it in female karakul sheep probably was because of their smaller muscle mass as compared to males (Alex and Laverne, 1983).

TBARS and FRAP a measure of lipid peroxidation and antioxidant status respectively have been also analysed in Karakul sheep to evaluate the metabolic challenges and maintenance of homeostasis. There are no reference values for these parameters available in the literature for sheep till date. The values recorded in Karakul sheep during present study are presented in Table 2. Gender bias was observed in TBARS with significantly ($P < 0.01$) higher values in males than females, however, no significant difference was recorded for FRAP between sexes. Higher levels of TBARS in males as compared to females suggest higher lipid peroxidation in males and as such males probably remain at higher risk of oxidative stress than females. TBARS and FRAP have now been extensively studied to assess the oxidative stress which provides a good reference of animal homeostasis. Evaluation of oxidative stress has contributed increasingly to our knowledge of the fundamental mechanisms involved in metabolic challenges, especially important in domestic animals in which many physiological process like lactation, pregnancy impose physiological demands on animal homeostasis (Castillo *et al*., 2006).

CONCLUSION

Present study in Karakul sheep did report gender bias in many haemato-biochemical parameters besides providing base values for various blood parameters in this breed of sheep. The present findings in karakul sheep could prove valuable resources in interpretation of clinical data and aid in livestock health care.

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REFERENCES

Addass, P.A., Perez, I.K.A., Midau, A., Lawan, A.U. and Tizhe, M.A. 2010. Haemato-biochemical findings of indigenous sheep breeds in Mubi Adamawa State, Nigeria. *Glob. Vet*., **4**(2): 164-167.

Aleksandar, V., Ana, S.V., Lada, R., Masa, E., Jadranka, P., and Miljenko, S. 2011. Hematological and biochemical reference intervals in Dalmatian pramenka sheep estimated from reduced sample size by bootstrap resampling. *Vet. Arh.*, **81**(1): 25-33.

Alex, K. and Laverne, L.S. 1983. Clinical chemistry; interpretation and Techniques. 2nd Ed., Washington, pp. 156-339.

Asakawa, T. and Matsushita, S. 1980. Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. *Lipids*, **15**: 137-140.

Benzie, I.F.F. and Strain, J.J. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of ''antioxidant power'' The FRAP Assay. *Anal. Biochem.*, **239**: 70–76.

Castillo, C., Hernandez, A.J., Valverde, I., Pereira, V., Sotillo, J., Lopez Alonso, M. and Benedito, J.L. 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. *Res. Vet. Sci.*, **80**: 133–139.

Chiranjib, S., Chittar, R.P., Subhasish, S., Anuradha, K., Archana, S. and Niranjan, B. 2015. Effect of prepartum concentrate supplementation on blood biochemical profiles of native ewes in coastal Odisha. *J. Anim. Res*., **5**(4): 813-817.

Coles, E.H. 1986. Veterinary Clinical Pathology. 4th Ed., W.B. Saunders Company, Philadelphia.

Devendran, P., Jayachandran, S., Visha, P., Nanjappan, K. and Panneerselvam, S. 2008. Hematology and blood profile of Coimbatore sheep. *Indian J. Small Rumin*., **15**: 98–101.

Douglas, J.W. and Wardrop, K. 2010. Schalm's Veterinary Haematology. 6th Ed., Wiley Blackwell Publishing Ltd. State Avenue, Ames, Iowa, USA.

Dutta, J.C., Baruah, R.N., Dutta, L. and Talukar, S.C. 1988. Blood biochemical studies in anoestrus and normal cyclic cattle. *Indian Vet. J*., **65**: 239–41.

Egbe-Nwiyi, T.N., Nwaosu, S.C. and Salami, H.A. 2000. Hematological values of apparently healthy sheep and goats as influenced by age and sex in Arid zone of Nigeria. *Afr. J. Biomed. Res*., **3**: 109-115.

Giltay, E.J., Fonk, J.C., Von Blomberg, B.M., Drexhage, H.A., Schalkwijk, C. and Gooren, L.J. 2000. In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. *J. Clin. Endocrinol. Metab.*, **85**: 1648–1657.

Jawasreh, K., Awawdeh, F., Bani, I.Z, Al-Rawasreh, O. and Al-Magali, A. 2010. Normal hematology and selected serum biochemical values in different genetic lines of Awassi Ewes in Jorden. *Int. J. Vet. Med*., **7**: 124–129.

Kaneko, J.J. 1989. Clinical biochemistry of domestic of animals. 4th Ed., Academic Press Inc., San Diego.

Keser, O. and Bilal, T. 2008. Effect of different dietary crude protein levels on performance, N digestibility and some blood parameters in kivircik lambs. *Acta. Veterinaria. (Beograd)*, **58**: 87-498.

Khan, K.H., Ahmed, K.A., Ahmad, E.H. and Omar, C.A. 2013. Study of some serum biochemical parameters of Karadi sheep in Sulaimani city, Iraq. *Res. Opin. Anim. Vet. Sci.*, **3**: 443-446.

Koch, R.M., Gregory, K.E., Ingalls, J.E. and Arthaud, R.L. 1959. Evaluating the Influence of sex on birth weight and preweaning gain in beef cattle. *J. Anim. Sci*., **18**:738.

Mabruka, S.S. 2014. Effect of gender on some plasma biochemical parameters of sheep from Southern Al Jabal Al Akhdar in Libya. *J. Am. Sci.*, **10**(8): 74-77.

Miloslav, S., Broucek, J. and Srejberova, P. 2011. Hematology and blood microelements of sheep in south Bohemia. *Biologia*, **66**: 181–186.

Mostaghni, K., Badiei, K. and Emadi, M. 2005. Hematology and serum biochemistry of captive wild sheep (*Ovisorientalisesphahanica*) in Iran. *Comp. Clin. Path*., **13**: 158-161.

NseAbasi, N., Etim, I., Mary, E., Williams, I., Uduak, A., Edem, E.A. and Offiong, I. 2014. Haematological parameters and factors affecting their values. *Agric. Sci.*, **2**: 37- 47.

Pampori, Z.A. and Pandita, S. 2013. Gender and age bias in immune response mediators and oxidative stress markers in Murrah buffaloes (*Bubalusbubalis*). *Indian J. Anim. Sci.*, **83**: 717-726.

Perez, J.M., Gonzalez, F.J., Granados, F.J., Perez, M.C., Fandos, P. and Sorigner, R.C. 2003. Haematologic and biochemical reference intervals for Spanish ibex. *J. Wild Dis*., **39**: 209–215

Rabee, A.S.O., Araz, O.B. and Hawar, M.H.Z. 2014. Factors affecting some hematology and serum biochemical parameters in three indigenous sheep breeds. *Adv.. Life Sci. Tech.*, **21:** 56-63

Rajput, Z.I., Song-hua, H.U., Arijo, A.G., Habib, M. and Khalid, M. 2005. Comparative study of anaplasma parasites in tick carrying buffaloes and cattle. *J. of Zhejiang Univ. of Sci*., **6:** 1057-1062.

Ramzan, M., Akhtar, M., Muhammad, F., Hussain, I., Hiszczynska-Sawicka, E., Haq, A.U., Mahmood, M.S. and Hafeez, M.A. 2009. Seroprevalence of Toxoplasma gondii in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. *Trop. Anim. Health. Prod.*, **41:** 1225-29.

Research Animal Resource. 2009. Reference values for laboratory animals: Normal haematological values. RAR Websites, RAR, University of Minnesota. Retrieved from http://www.ahc.umn.edu/rar/refvalues.html.

Roubiens, N., Panousis, N., Fytianou, A., katsoulos, P.D., Giainis, N. and Karatzias, H. 2006. Effect of age and reproductive stage on certain serum biochemical parameters of Chios sheep under greek rearing conditions. *J. Vet. Med*., **53**: 277-281.

Saad I. Al-Sultan and Sabry, M.E. 2014. Effect of urea on haematological and selected biochemical parameters of growing Somali lambs. *J. Anim. Res*., **4**(1): 65-73.

Shumaila, K., Arif, M.B., Bakhtyawar, A.K., Sobia, D., Muhammad, A., Muhammad, A., and Furhan, I. 2012. Effect of age and gender on some blood biochemical

parameters of apparently healthy small ruminants from Southern Punjab in Pakistan. *Asian Pac. J. Trop. Biomed.*, **2**(4): 304–306.

Snedecor, G.W. and Cochran, W.G. 1982. Statistical Methods. 6th Ed., Iowa State University Press, USA.

Tambuwal, F.M., Agale, B.M. and Bangana, A. 2002. Haematological and serum biochemical values of apparently healthy red Sokoto goats. *Proc. of 27th Annual Conference of Nigerian Society of Animal Production*, pp. 50-53.